Influence of Propylene Glycol Buffers on the Stability of Red Cabbage Anthocyanins

Research thesis presented in partial fulfillment of the requirements for graduation with Research Distinction in the undergraduate colleges of The Ohio State University

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ABSTRACT

Although synthetic colorants have been widely used for decades, recent consumer trends have caused increased interest in developing natural colorants from anthocyanins. Despite their potential to produce a wide variety of colors, they are restricted in their use as colorants because of their instability in retaining color. Our objective was to determine how a common water activity reducing additive in the food industry, propylene glycol, affected the stability of anthocyanins in different buffer solutions. Non-acylated, mono-acylated, and di-acylated anthocyanins were extracted from red cabbage (*Brassica oleracea var.* capitata f. rubra) and pigment samples were combined with buffers made up of varying percentages of propylene glycol. The anthocyanin concentration and performance of the samples were analyzed with SpectraByColor software and a Shimadzu spectrophotometer. Results indicated that anthocyanin intensity appeared to decrease in propylene glycol, suggesting that anthocyanin hydration occurred even with very low water levels. Contrary to the hypothesis that increasing propylene glycol content and lowering water activity would decrease the hydration of anthocyanins and increase the color intensity of the pigments, it was observed that increasing propylene glycol (<100%) resulted in an increasing loss of pigment intensity. This experiment can help to determine the type of additives necessary for optimal performance of anthocyanins in commercial products without synthetic colorants, and to encourage future explorations in developing natural food colors that are more pigmented and more stable than what are currently used.

INTRODUCTION

Growing consumer trends towards natural food preferences have raised interest in developing naturally derived food colorants to replace the commonly used artificial colorants. Anthocyanins are flavonoid compounds that absorb visible light, differentiating them from other flavonoids and are able to produce a wide variety of colors (Markakis 1982). Because of their ability to express a wide range of hues, they are being investigated as potential alternatives to replace artificial colors. However, anthocyanins are less stable than their artificial counterparts, thus creating a need to study means to enhance anthocyanin stability and methods to maintain the pigments' color intensities.

Factors such as pH, chemical structure, and water activity affect the stability and pigment retention of anthocyanins. Change in pH not only impacts the color of the anthocyanin but also affects their degradation. When anthocyanins are in acidic conditions, red hues are observed. The red hues are lost and the pigments become colorless in mildly acidic pH (prevalent to foods) and then they express purple-blue colors as the pH becomes more basic. Anthocyanins with added acyl groups in its chemical makeup (acylation) can affect the stability of the anthocyanin as the pH goes from acidic to neutral, increasing pigment retention (Wrolstad and Culver 2012). Some research expressed that pigments with diacylation are more stable than those with monoacylation and no acylation (Brouillard 1983, Rodriguez-Saona, Giusti, & Wrolstad 1999).

Other research has indicated that increasing water activity causes an increase in anthocyanin degradation. Because water affects the stability of anthocyanins and discolors the pigments over time, lowering water activity may help the anthocyanins maintain their color (Garzon & Wrolstad 2001). One such water binding agent is sucrose, which research indicated that anthocyanins derived from roselles increased their stability in sucrose solutions due to less free water (Tsai, Hsieh & Huang, 2004).

Similar to sucrose, propylene glycol is often used by chemical, pharmaceutical, and food industries to reduce water activity, making it another possible solution to the stabilization of anthocyanin pigments (Markakis 1982). In the food industry, the use of artificial colors is commonly found in high sugar products such as candy, so propylene glycol may be the more cost-effective additive as it has a higher ranking of sweetness than sucrose (Moskowitz 1970).

The focus of this study was to examine the ability of propylene glycol solutions in increasing the stabilization of anthocyanins derived from red cabbage in different pHs. Red cabbage was used in this study because it is a commercially available colorant, and therefore it is a probable candidate for future widespread industry use. The ability to reduce water activity in solutions of red cabbage anthocyanins and propylene glycol is an important investigation if the food industry continues to develop and expand methods of natural food colorants.

HYPOTHESIS

It is hypothesized that increasing the percentages of propylene glycol will increase the stability of anthocyanins. Because water activity lowers as the amount of propylene glycol increases, the anthocyanins will be able to retain their color for a longer period of time.

MATERIALS AND METHODS

From the extracts of red cabbage, non-acylated, mono-acylated, and diacylated anthocyanins were isolated and purified using prep-HPLC. The concentration of the anthocyanin extracts were determined by absorbance in pH 1 and using the molar absorptivities determined by Ahmadiani et al 2015.

The concentrated anthocyanin extracts were diluted to 50 μM in different buffer-propylene glycol solutions. The buffer solutions were prepared at pHs of 4, 6, and 8 using 0.1 M concentrations of TRIS and sodium acetate. The pH levels of 4 and 6 were used because anthocyanins have high degree of hydration and color loss at those levels, and pH 8 was used due to the poor stability of anthocyanins.

Small aliquots of the 0.1 M buffers were then combined with the propylene glycol buffers to obtain aqueous solutions of 20, 40, 60, and 80% concentrations. Samples were read by spectrophotometry for absorption between 380 to 700 nm. After initial readings, samples were stored in glass vials in the dark at 25 °C and read one and two weeks out from the initial reading. Absorbance, $\lambda_{vis\text{-}max}$ was compared from the spectrometric data; colorimetric data was collected from spectral absorbance data using SpectraByColor software (Farr and Giusti 2017).

RESULTS AND DISCUSSION

After reviewing the data produced by the spectrophotometer, it was observed that increasing the percentages of propylene glycol within each pH category did not enhance the color pigmentation of the anthocyanins (Figure 1). Increasing the percentage amount of propylene glycol (<100%) appeared to visually result in an increasing loss of color intensity (indicating an increase or no effect on the hydration of the anthocyanins) instead of the hypothesized improved color expression and decrease in dehydration.

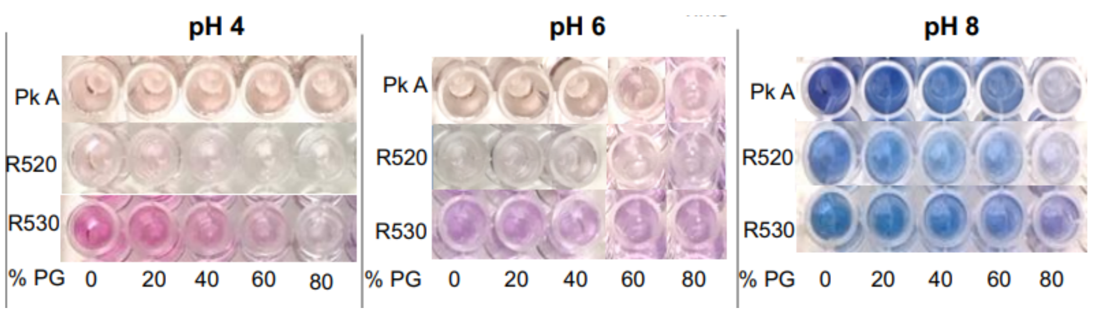

Figure 1. Expression of anthocyanin pigments in propylene glycol solutions at different percentages and pHs

The measurements of absorbance, $\lambda_{vis\text{-}max}$, also showed the loss of color intensity with the increasing propylene glycol amounts, as indicated by the decreasing trend among all three anthocyanin extracts against the increasing amounts of propylene glycol (Figure 2). Under pH 6, there was a slight increase in absorbance readings at 80% propylene glycol which may have occurred due to confounding external variables not studied in this experiment.

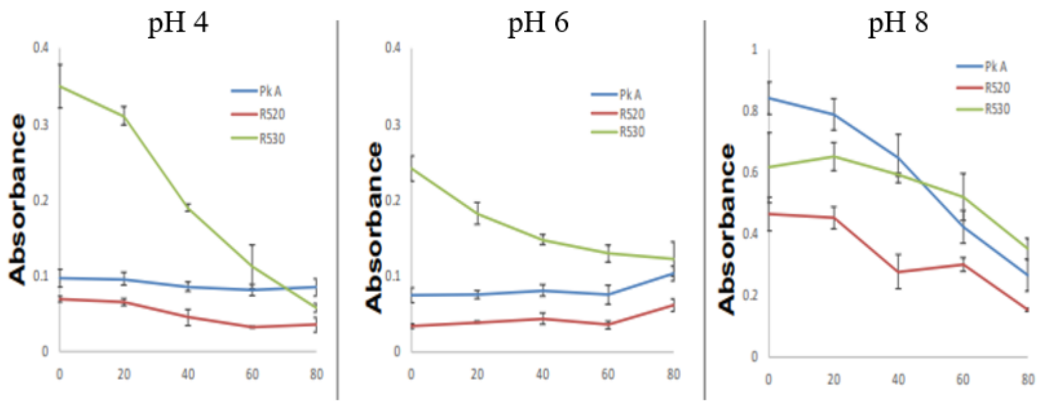

Figure 2. Trends correlating absorbance and percentage of propylene glycol in buffer solutions

Overall the data suggested that very low water levels are enough for hydration to occur, which resulted in color loss. Further research should investigate the amount of water needed to cause color loss, or perhaps to evaluate the effects of propylene glycol on pigment expression.

CONCLUSION

Though anthocyanins are the probable future of natural food colorings, research must be done to further understand color stability and retention. Extracted non-acylated, mono-acetylated, and diacylated anthocyanins were used to learn how lowering water activity may improve pigment retention and increase stability. Overall, minimal improvements in pigment retention of anthocyanins in propylene glycol solutions were observed, however this information can help future research in defining the amount of water needed to cause color loss and assist explorations in developing natural food colors that are more pigmented and more stable than what are currently used.

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